A cost-effective approach to microporate mammalian cells with the Neon Transfection System

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Abstract

Electroporation is one of the most efficient non-viral methods for transferring exogenous DNA into mammalian cells. However, the relatively high costs of electroporation kits and reagents temper the routine use of this fast and easy to perform technique in many laboratories. Several years ago, a new flexible and easy to operate electroporation device was launched under the name "Neon™ Transfection System". This device uses specialized pipette tips containing gold-plated electrodes as electroporation chamber. Here we report a protocol to regenerate these expensive tips as well as some other Neon™ kit accessories, thereby reducing the cost of electroporation at least ten-fold.

Keywords

Transfection; Electroporation; Microporation, Mammalian cells; Plasmid DNA; DNase I

Many cell biology experiments require the introduction of genetic material into cells. A variety of viral and non-viral delivery systems have been developed, but electroporation is often the selected method of choice [1]. The underlying reason is that this method, which employs electrical pulses to create transient pores in cell membranes, reproducibly yields high transfection efficiencies in combination with low cell mortality in a wide range of cell lines [2]. However, a major drawback limiting its broad utilization, especially in less-well financed research groups, is the high and ongoing cost associated with the purchase of the electroporation kits. Here we present a simple and cost-saving approach to electroporate mammalian cells with the Neon™ Transfection System (Invitrogen), a device that is increasingly gaining in popularity.

The Neon™ System employs specialized, consumable pipette tips containing gold-plated electrodes as electroporation chamber. In general, the use of such a capillary electroporation system (which is often called a microporation device) has important advantages compared to conventional cuvette-based electroporation chambers: (i) the method is rapid, reproducible, and free of cell loss, (ii) the overall cell viability is higher, and (iii) the transfection efficiencies are superior, even for hard-to-transfect cells [3]. However, to ensure repeatability and eliminate variation of the transfection conditions, the supplier strongly advices users not to employ Neon™ pipette tips more than two times. In addition, the supplier does not provide a protocol to regenerate these tips or any other component of the electroporation kit (e.g. the Neon™ tubes, which hold the electrolytic buffer during electroporation) so that they can be re-used later on to perform high quality transfections with different plasmids. To overcome this problem, we have developed a fast, reliable, and inexpensive protocol to regenerate Neon™ pipette tips and tubes for multiple re-use. We found that the Neon™ pipette tips and tubes can be recycled at least ten

times without significant loss in transfection efficiency (Fig. 1A). In addition, no cross-contamination from the previous plasmid preparation could be detected in transfected cells (Fig. 1B).

Here, we provide a protocol for recycling Neon™ pipette tips and tubes. Note that Neon™ kits are available for electroporation of 10 µl or 100 µl samples, and that our protocol has been developed with the 10 µl tips. However, by adjusting the volume amounts, the approach should also work for the 100 µl tips. After electroporation, the used tips and tubes are collected and stored at room temperature until regeneration (normally once a week). To regenerate the tips, residual plasmid DNA is first removed by pipetting three times 10 µl of a DNase I solution (1 mg/ml DNase I in phosphate-buffered saline (PBS) containing 5 mM $MgCl_2$, pH 7.4) and subsequently incubating the tips for 15 to 20 minutes at room temperature. Next, the tips are rinsed by pipetting first 10 µl of sterile water (3x) and thereafter 10 µl of 70% (v/v) ethanol (3x). Finally, the tips are air-dried in a sterile laminar flow hood. To regenerate the tubes, they are first thoroughly washed with distilled water, thereafter rinsed with 70% (v/v) ethanol, and finally also air-dried in a sterile laminar flow hood. The recycled materials can subsequently be used for electroporation according to the procedures recommended by the manufacturer. Importantly, the limited amounts of the patented resuspension and electrolytic buffers that are provided with the electroporation kit and of which the composition is not specified, can be easily replaced by a filter-sterilized sucrose-based buffer, called SBB (250 mM sucrose and 1 mM $MgCl_2$ in Dulbecco's PBS (DPBS; Gibco cat. no. 14190)) [4], without effecting transfection efficiency (data not shown; the results shown in Fig. 1A were obtained with home-made SBB).

Based on our experience, the Neon™ tips and tubes can be regenerated at least 10 times with little or no deterioration in performance (Fig. 1A). In addition, it is worthwhile to note that the described procedure is already routinely carried out in our laboratory for several years, and that other laboratories have expressed their interest in using this easy and effective Neon™ kit component recycling protocol to perform microporation experiments at affordable costs.

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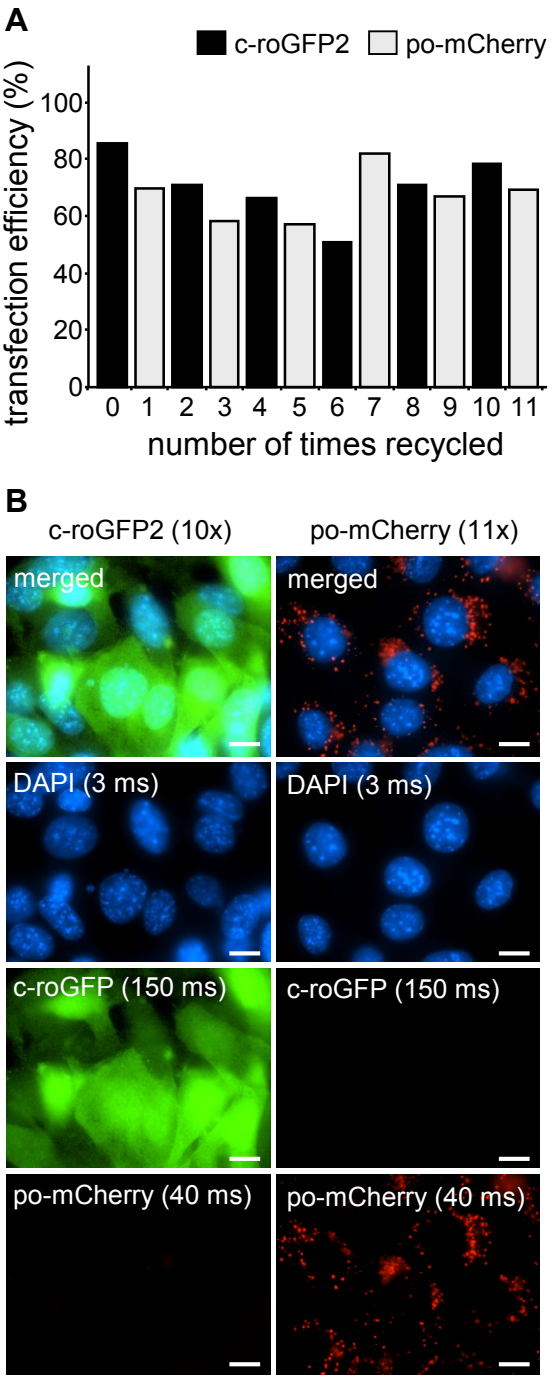
Figure legend

Fig. 1. Influence of Neon^{TM} pipette tip and tube regeneration on transfection efficiency. Immortalized mouse embryonic fibroblasts were microporated (settings: 1350 V pulse voltage, 30-ms pulse width, 1 pulse; resuspension buffer: SBB; electrolytic buffer: SBB) with a plasmid encoding a cytosolic variant of the redox-sensitive enhanced green fluorescent protein (c-roGFP) or a peroxisomal variant of the red fluorescent protein mCherry (po-mCherry). To assess potential cross-contamination in sequential experiments, the two plasmids were used in alternate order. After electroporation, the cells were cultured in standard growth medium for two days, fixed, counterstained with DAPI (to visualize nuclei), and processed for fluorescence microscopy as described elsewhere [5]. (A) Effect of pipette tip and tube recycling on transfection efficiency. To monitor the transfection efficiency, the percentage of cells expressing c-roGFP2 or po-mCherry was determined by counting on average 135 randomly-selected cells. (B) Neon^{TM} pipette tips and tubes can be recycled without a risk of cross-contamination. Representative fluorescence images of cells microporated with pipette tips and tubes that are used for the 10th or 11th time. The image acquisition times are indicated in milliseconds (ms). Scale bar: 10 μm.

Fig. 1


A
c-roGFP2
po-mCherry
transfection efficiency (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11
number of times recycled
B
c-roGFP2 (10x)
po-mCherry (11x)
merged
merged
DAPI (3 ms)
DAPI (3 ms)
c-roGFP (150 ms)
c-roGFP (150 ms)
po-mCherry (40 ms)
po-mCherry (40 ms)